

WHAT IS CLAIMED IS:

1. An amplification primer pair comprising an oligonucleotide anchor and primer, said anchor having a nucleic acid chemistry which is not a substrate for reverse transcriptases or DNA polymerases, and/or having a 3'-end which is not  
5 capable of priming nucleic acid synthesis;

wherein said primer has a nucleic acid chemistry that is a substrate for reverse transcriptases or DNA polymerases; and

wherein said anchor and said primer each include a region of complementary nucleotides which readily associate with each other to form a stem structure in the  
10 absence of a target nucleic acid, wherein the stem structure includes a region which is complementary to a universal primer.

2. The primer pair of Claim 1, wherein said anchor sequence and said stem regions are connected by a flexible linker.

3. The primer pair of Claim 2, wherein said flexible linker is selected  
15 from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

4. The primer pair of Claim 1, wherein said primer comprises a tail region which extends beyond the length of said stem region of said anchor.

5. The primer pair of Claim 1, wherein said primer comprises one or more  
20 modified bases.

6. The primer pair of Claim 1, wherein said anchor comprises one or more modified backbone linkages.

7. The primer pair of Claim 1, wherein said anchor and said primer are each between 6 and 24 bases in length.

8. The primer pair of Claim 1, further in association with a universal  
25 primer which is complementary to a region of said stem structure.

9. A sequencing primer pair comprising an oligonucleotide anchor and primer, said anchor having a nucleic acid chemistry which is not a substrate for reverse transcriptases or DNA polymerases, and/or having a 3'-end which is not  
30 capable of priming nucleic acid synthesis;

wherein said primer has a nucleic acid chemistry that is a substrate for reverse transcriptases or DNA polymerases; and

wherein said anchor and said primer each include a region of complementary nucleotides which readily associate with each other to form a stem structure in the absence of a target nucleic acid.

10. The primer pair of Claim 9, wherein said stem structure includes a region which is complementary to a universal primer.

11. The primer pair of Claim 10, wherein said anchor sequence and said stem region are connected by a flexible linker.

12. The primer pair of Claim 11, wherein said flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

13. The primer pair of Claim 10, wherein said primer comprises a tail region which extends beyond the length of said stem region of said anchor.

14. The primer pair of Claim 9, wherein said primer comprises one or more modified bases.

15. The primer pair of Claim 9, wherein said anchor comprises one or more modified backbone linkages.

16. The primer pair of Claim 9, wherein said anchor and said primer are each between 6 and 24 bases in length.

17. The primer pair of Claim 9, further in association with a universal primer which is complementary to a region of said stem structure.

18. A method for amplifying a target nucleic acid sequence, comprising the steps of: combining said target nucleic acid sequence a forward anchor (FA), forward primer (FP), reverse anchor (RA), reverse primer (RP), forward universal primer (FUP) and reverse universal primer (RUP), wherein said FA/FP readily associate to form a first primer pair and said RA/RP readily associate to form a second primer pair via association of their complementary stem regions in the absence of said target nucleic acid, wherein said FUP is complementary to the FA/FP stem region, and wherein said RUP is complementary to the RA/RP stem region wherein said primer

pairs are selected on the basis of complementarity to said target nucleic acid sequence to flank said target nucleic acid sequence; and

amplifying said nucleic acid sequence via enzyme-mediated amplification.

19. The method of Claim 18, wherein said nucleic acid sequence encodes a therapeutic gene product.

20. The method of Claim 18, wherein said nucleic acid sequence is DNA or RNA.

21. The method of Claim 18, wherein said enzyme-mediated amplification is PCR amplification.

22. A method for amplifying a target nucleic acid sequence, comprising:  
providing a first oligonucleotide primer comprising a first target binding region configured to bind to a first end of said target nucleic acid sequence, and a first stem region, wherein said first stem region comprises a first universal primer binding region;

15 providing a second oligonucleotide primer comprising a second target binding region configured to bind to said first end of said target nucleic acid sequence, and a second stem region that is homologous with said first stem region, so that said first oligonucleotide primer and said second oligonucleotide primer join together to form a first primer pair that binds to  
20 said target nucleic acid sequence;

providing a universal primer configured to bind with said first universal primer binding region;

providing an additional primer configured to bind with a second end of said target nucleic acid sequence; and

25 incubating said first primer pair, universal primer, and additional primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid.

23. The method of Claim 22, wherein the first stem region and the first target binding region are connected by a flexible linker.

24. The method of Claim 23, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.
25. The method of Claim 22, wherein the second stem region and the second target binding region are connected by a flexible linker.
26. The method of Claim 25, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.
27. The method of Claim 22, wherein the first oligonucleotide primer comprises one or more modified bases.
28. The method of Claim 22, wherein the second oligonucleotide primer comprises one or more modified bases.
29. The method of Claim 22, wherein the target nucleic acid sequence is DNA.
30. The method of Claim 22, wherein the target nucleic acid sequence is RNA.
31. The method of Claim 22, wherein said incubating comprises the Polymerase Chain Reaction.
32. The method of Claim 22, wherein said amplifying is mediated by an enzyme selected from the group consisting of: Taq polymerase and reverse transcriptase.
33. The method of Claim 22, wherein said additional primer comprises:  
a third oligonucleotide primer comprising a third target binding region configured to bind to a second end of said target nucleic acid sequence, a reverse transcriptase binding region, and a third stem region, wherein said third stem region comprises a second universal primer binding region; and  
a fourth oligonucleotide primer comprising a fourth target binding region configured to bind to said second end of said target nucleic acid sequence, and a fourth stem region that is homologous with said third stem region, so that said third oligonucleotide primer and said fourth

oligonucleotide primer join together to form a second primer pair that binds to said target nucleic acid sequence.

34. A method of amplifying a target nucleic acid sequence, comprising the steps of:

5                    providing a target nucleic acid sequence having a first amplification region and a second amplification region;

                  selecting from a library of first oligonucleotide primers, a first oligonucleotide primer that comprises a first target binding region that is homologous with a portion of the first amplification region, wherein said first  
10                   oligonucleotide primer comprises a first stem region and a first universal primer binding region;

                  selecting from a library of second oligonucleotide primers, a second oligonucleotide primer that comprises a second target binding region that is homologous with a second portion of the first amplification region, wherein  
15                   said second oligonucleotide primer comprises a second stem region that is homologous with said first stem region, so that said first oligonucleotide primer and said second oligonucleotide primer join together to form a first primer pair that binds to said target nucleic acid sequence at said first amplification region;

20                   providing a universal primer configured to bind with said first universal primer binding region;

                  providing an additional primer configured to bind with a second amplification region of said target nucleic acid sequence; and

                  incubating said first primer pair, universal primer, and additional  
25                   primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid.

35. The method of Claim 34, wherein the library of first oligonucleotide primers comprises greater than 65,536 oligonucleotides.

36. The method of Claim 34, wherein the first target binding region  
30                   comprises at least six nucleotides.

37. The method of Claim 34, wherein the second target binding region comprises at least six nucleotides.

38. The method of Claim 34, wherein the first target binding region and the first stem region are connected by a flexible linker.

5           39. The method of Claim 38, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

40. The method of Claim 34, wherein the second target binding region and the second stem region are connected by a flexible linker.

10           41. The method of Claim 40, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

42. The method of Claim 34, wherein the first oligonucleotide primer comprises one or more modified bases.

15           43. The method of Claim 34, wherein the second oligonucleotide primer comprises one or more modified bases.

44. The method of Claim 34, wherein the first oligonucleotide primer comprises one or more modified backbone linkages.

20           45. The method of Claim 34, wherein the second oligonucleotide primer comprises one or more modified backbone linkages.

46. The method of Claim 34, wherein the target nucleic acid sequence is DNA.

47. The method of Claim 34, wherein the target nucleic acid sequence is RNA.

25           48. The method of Claim 34, wherein said incubating comprises the Polymerase Chain Reaction.

49. The method of Claim 34, wherein said amplifying is mediated by an enzyme selected from the group consisting of Taq polymerase and reverse transcriptase.

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